

GLUCOSE-6-PHOSPHATE ISOMERASE VARIATION AND GENETIC
STRUCTURE IN *YUCCA BREVIFOLIA* (AGAVACEAE)

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ABSTRACT

We used electrophoretic analysis of isozymes to investigate genetic structure of the Mojave Desert endemic, *Yucca brevifolia*. To test the hypothesis that, because of geographic isolation, most variation in allele frequencies would be between populations we sampled three subpopulations in each of five distinct populations: Arizona, Joshua Tree, Mojave, Western Mojave, and Utah. Two of nine proteins had isozymes that resolved adequately. One of these, Superoxide dismutase (SOD) was monomorphic. For the other, glucose-6-phosphate isomerase (GPI), five alleles were identified, and the three most common alleles were present in all populations sampled. For GPI, allele frequencies differed significantly between all five populations, as well as between subpopulations in both the Joshua Tree and Arizona populations.

We also tested the hypothesis that there would be strong localized genetic structure due to the high proportion of recruitment attributed to vegetative reproduction. Based on GPI data from an intensively sampled 1 ha plot, genotypes were randomly distributed, and thus sexual reproduction through outcrossing may be the principal mode of recruitment in *Y. brevifolia* at this site.

This study describes genetic structure within populations of *Y. brevifolia* based on protein variation at a single locus. Future research, using additional markers (nuclear, cytoplasmic, or both) is necessary to understand the dynamics of gene flow, genetic variation, and recruitment within the species *Y. brevifolia*.

Key Words: Genetic structure, glucose-6-phosphate isomerase, Mojave Desert, spatial autocorrelation, *Yucca brevifolia*.

The distribution of *Yucca brevifolia* Engelm., an endemic of the Mojave Desert, includes portions of California, Nevada, Utah, and Arizona. Within this distribution, the Joshua tree is found in isolated populations at altitudes between 600 and 2200 m. The limits of the species distribution, both altitudinally and latitudinally, appear to be a result of sensitivity to mean temperature maxima and minima (Gates 1966).

These temperature limitations produced altitudinal and latitudinal changes in the distribution of the species over the geologic time scale as a result of climatic variation. Evidence of altitudinal range contractions has been observed in both the Panamint mountain range and Death Valley, where *Y. brevifolia* tissue has been identified in packrat middens from the Pleistocene era at elevations more than 125 m lower than the current distribution (Grayson 1993). Changes in latitudinal distribution are demonstrated by *Yucca brevifolia* tissue identified in middens near the Mexican border approximately 240 km South of the species current limit (Laudermilk and Munz 1934; Grayson 1993).

The current distribution of *Y. brevifolia*, comprised of isolated populations limited to the

Mojave Desert, has likely had strong effects on genetic variation within and among populations. Genetic studies of both fragmented and endemic plant species typically reveal limited genetic variation within populations, while genetic differentiation among these populations tends to be high as a result of genetic drift and natural selection (Chung et al. 2004; Fracaro and Echeverrigaray 2006; Meister et al. 2006; Zhao et al. 2006). In addition to the geographic distribution of a species, genetic variation within populations is also influenced by factors including pollination, rates of inbreeding, outcrossing, and asexual reproduction (Loveless and Hamrick 1984).

Methods of recruitment within *Y. brevifolia* populations are poorly understood. *Yucca brevifolia* is pollinated by the moth species *Tegiticula synthetica* and *T. antithetica*, but it is unknown whether, in addition to moth pollination, the flowers undergo self-pollination (Pellmyr and Segraves 2003). Further, the relative importance of sexual and asexual reproduction in populations is also unknown. Clonal populations have been reported in southern California (Boyd 1999) and populations in other areas may also exhibit vegetative reproduction. While a high proportion of sexual reproduction through random mating would result in a random distribution of genotypes in a population, a high proportion of vegetative reproduction and/or inbreeding would

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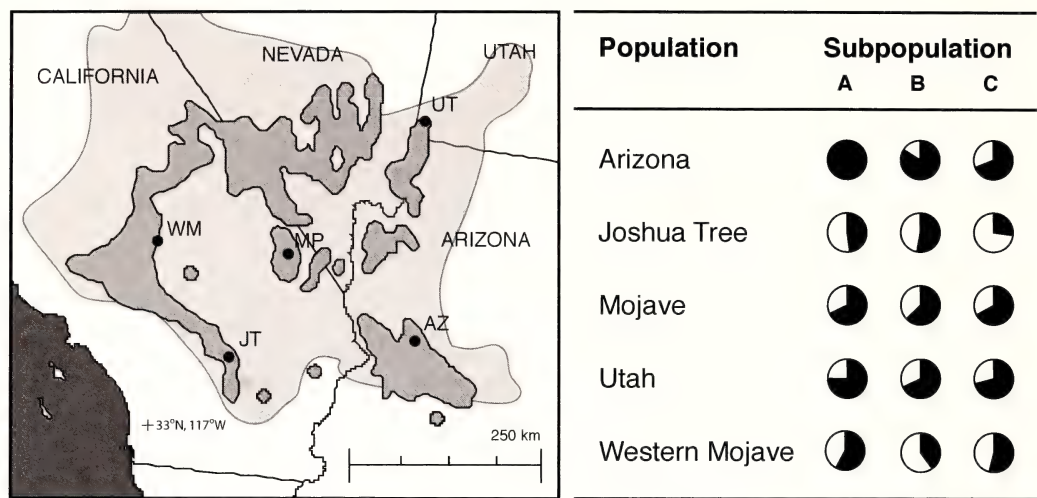


FIG. 1. Map of populations sampled in this study with Mojave Desert boundaries indicated by light grey and the distribution of *Y. brevifolia* overlaid in dark grey (Little 1976; Griffiths et al. 2006). Pie charts depict the frequency of the most common allele, GPI 3, in each subpopulation.

result in localized genetic structure. For a general discussion of the biology of *Yucca brevifolia* see Gucker (2006).

The present study employs glucose-6-phosphate (GPI) allozymes to investigate genetic structure in *Y. brevifolia*. This enzyme, coded for by a single locus in *Y. brevifolia*, is highly polymorphic, so it is well suited as a marker to study outcrossing, inbreeding and cloning. However, geographic variation in GPI allele frequencies may result from selection (Gillespi 1991), which could confound the effects of geographic isolation on gene flow and genetic drift. Therefore, geographic variation in GPI allele frequencies may reflect some combination of isolation, selection, and drift. Markers that are both highly variable and neutral have yet to be identified in *Y. brevifolia*.

Regarding the genetic structure of *Y. brevifolia* we test the following hypotheses: First, allele frequency would vary significantly among geographically isolated populations. Second, allele frequencies would be homogenous among subpopulations since moth aided pollination and seed dispersal by animal and wind should lead to unrestricted gene flow within populations. Third, that outcrossing is the predominant form of sexual reproduction within *Y. brevifolia*. And finally, that recruitment through vegetative reproduction occurs at levels sufficient to result in spatial structuring within some populations.

METHODS

Study Sites

We selected five populations for sampling that spanned the distribution of *Y. brevifolia* including

a large central population found in the Mojave Preserve (MP) and four peripheral populations; Joshua Tree National Park (JT), Arizona (AZ), Utah (UT) and Western Mojave (WM). Three of the populations were disjunct (AZ, MP, UT) while two populations were contiguous (JT, WM). All populations were separated from one another by a minimum of 200 km (Fig. 1, Table 1). Both disjunct and nondisjunct populations were included in this study to permit assessment of long distance gene flow between contiguous populations. The populations included both var. *brevifolia* (AZ, JT, WM) and var. *jaegeriana* (MP, UT).

Within each of these five populations, three subpopulations were sampled that were a minimum of 1.6 km apart. Subpopulation samples consisted of 48 trees, except for the Western Mojave subpopulations where low density and restricted access limited sample sizes to 24 trees per subpopulation, selected haphazardly. Trees were selected from all size classes and were at least 10 m apart. From each tree, three recently developed leaves were collected and stored on dry ice.

In addition to the five populations, we also intensively sampled a 1-ha plot in Joshua Tree National Park to assess localized genetic structure. In the plot, sampled stems were separated by at least 1 m. Where two or more stems were <1 m apart, only one stem was sampled. The location of each stem was recorded using a global positioning system (GPS) device (Trimble Navigation Limited, Sunnyvale, CA).

Electrophoresis

Leaves were ground in liquid nitrogen and Wendell and Parks grinding solution (Wendell

TABLE 1. SPATIAL SUMMARY OF SUBPOPULATIONS.

Location	UTM	Altitude (m)	Distance (km) from	
			Site A	Site B
AZ A	12S 237445, 3836618	911	—	—
AZ B	12S 245555, 3833080	983	7.4	—
AZ C	12S 214633, 3854886	552	29.0	37.0
JT A	11S 578989, 3763630	1373	—	—
JT B	11S 577799, 3760240	1322	2.0	—
JT C	11S 575000, 3767939	1313	6.0	7.4
MP A	11S 636014, 3902473	1318	—	—
MP B	11S 635602, 3896408	1190	6.3	—
MP C	11S 615813, 3890137	1114	23.2	19.0
UT A	12S 242593, 4104714	1095	—	—
UT B	12S 242389, 4103013	994	1.6	—
UT C	12S 241096, 4100972	885	3.9	2.4
WM A	11S 436849, 3874514	731	—	—
WM B	11S 431628, 3873700	713	4.0	—
WM C	11S 442307, 3873191	754	4.6	9.2

and Parks 1982) and centrifuged at 2000 g for 10 min. Filter paper wicks were dipped into the supernatant and loaded onto a 12% starch gel. The gels were electrophoresed at 75 volts until the tracking dye ran off the edge of the gel, usually after 5–6 hrs.

Initially, 23 individuals from across the five populations were surveyed to identify allozymes that resolved consistently and exhibited variation. Each of the 23 samples were stained for glucose-6-phosphate isomerase (GPI), phosphoglucomutase (PGM), glucose-6-phosphate dehydrogenase (G6PDH), isocitrate dehydrogenase (IDH), alcohol dehydrogenase (ADH), superoxide dismutase (SOD), malate dehydrogenase (MDH), peroxidase (PRX) and esterase (EST) using system 6 and 11 buffers (Soltis et al. 1983), Poulik (Poulik 1957), and LiOH, and TC8 (Selander et al. 1971). After this initial survey, all samples were run using the LiOH buffer system and stained and scored for GPI, the only enzyme that resolved consistently and exhibited polymorphism. For this portion of the experiment, mylar supported cellulosic gels (Semi-Micro II Chamber and Supra Sepraphore gels, Gelman Sciences, Ann Arbor, MI) were used instead of 12% starch gels after the same allelic patterns were observed using both systems. Alleles were numbered 1–5 based on position relative to the anode, with one being the most anodal.

Data Analysis

We used the online program Genepop (Raymond and Rousset 1995) for calculating the levels of heterozygosity within populations, testing for Hardy Weinberg equilibrium, and making pairwise comparisons of population differentiation. Where subpopulations were homogenous, they were pooled prior to performing pair-wise comparisons among populations

For the intensively sampled 1-ha plot, we performed spatial autocorrelation analysis using the Moran's I test of ArcView 3.2 (ESRI, Redlands, CA). We ran the Moran's I test under randomization of attribute (genotype), which samples attribute values without replacement. In addition, the Moran's I test employed only the smallest distance class observed between stems because that is the class most likely to exhibit spatial structuring.

RESULTS

Glucose-6-phosphate isomerase resolved as a single polymorphic locus with a total of five alleles detected in the populations studied. Of the five alleles, the three most common were observed in all populations, while all five were found only in the most central population, the Mojave preserve (Fig. 1, Table 2).

Levels of heterozygosity in subpopulations were variable with expected values ranging from 0.00 in AZA to a 0.62 in JTA (Table 2). The JT subpopulations exhibited the highest levels of heterozygosity across the subpopulations. All subpopulations were found to be in Hardy-Weinberg equilibrium (Hardy-Weinberg Exact Test [Haldane 1954], $P > 0.05$).

The results of pair-wise tests for homogeneity indicated that within population allele frequencies in MP, UT and WM were homogenous across subpopulations. However, significant heterogeneity was found among subpopulations at both JT and AZ (Table 3; $P < 0.05$). With the exception of three comparisons (AZC with JTB, $P = 0.06$; AZC with MPABC, $P = 0.65$; and JTB with MPABC, $P = 0.14$), all pairwise comparisons of allele frequencies across populations revealed significant heterogeneity ($P < 0.01$).

The expected heterozygosity of the intensively sampled one hectare plot was 0.65. The popula-

TABLE 2. ALLELE FREQUENCIES OBSERVED AT EACH SUBPOPULATION WITHIN THE FIVE SAMPLED POPULATIONS (MOST COMMON ALLELE IN BOLD) ALONG WITH THE EXPECTED HETEROZYGOSITY (H_E) FOR EACH SUBPOPULATION.

Pop.	Subpop.	Allele					H_E
		GPI 1	GPI 2	GPI 3	GPI 4	GPI 5	
AZ	A			1.00			0.00
	B			0.84	0.04	0.12	0.28
	C			0.69	0.18	0.13	0.47
JT	A		0.08	0.48	0.38	0.06	0.62
	B		0.01	0.53	0.32	0.14	0.60
	C		0.05	0.27	0.59	0.08	0.57
MP	A	0.01		0.68	0.25	0.06	0.47
	B			0.63	0.26	0.12	0.52
	C		0.01	0.67	0.19	0.13	0.50
UT	A	0.06		0.75	0.17	0.02	0.40
	B	0.07		0.68	0.20	0.05	0.49
	C	0.05		0.71	0.17	0.07	0.46
WM	A			0.58	0.40	0.02	0.50
	B			0.40	0.58	0.02	0.50
	C			0.54	0.44	0.02	0.51

tion was in Hardy-Weinberg equilibrium. Moran's I indicated a random distribution of genotypes across plants in the plot ($Z = -1.12$).

DISCUSSION

Genetic Structure

Our data are consistent with the hypothesis that geographic isolation among populations of *Y. brevifolia* restricts gene flow below the level required for homogenization of allele frequencies at the GPI locus. However, given that some studies have suggested an adaptive role for GPI variation (Gillespi 1991; Katz and Harrison 1997; Wheat et al. 2006), the variation in allele

frequencies may be a reflection of selection in this long-lived plant rather than the effects of geographic isolation between the five populations.

Our data also show heterogeneity in allele frequencies among subpopulations within both the AZ and JT populations. Clearly, our hypothesis that moth pollination coupled with wind and animal seed dispersal would produce sufficient gene flow to sustain homogeneity of allele frequencies across subpopulations is not supported. While subpopulation AZC is separated from both AZA and AZB by relatively large distances (29 km and 37 km respectively), such is not the case for AZA and AZB (7.4 km) or for the JT subpopulations (2–7.4 km). The AZ population is in the transition zone between the Mojave and Sonoran deserts in an area with a mix of communities associated with each desert. In addition, this population spans the largest altitudinal range (>400 m) and includes a subpopulation (AZC) 50 m below the reported elevation range of *Y. brevifolia*. The JT population however, is in a relatively homogeneous environment. Whether the observed variation in allele frequencies is a result of selection (as suggested by the environmental heterogeneity in AZ) or genetic drift (as suggested by the apparent environmental homogeneity in JT), pollen and seed dispersal in *Y. brevifolia* appears restricted, in some locations, even over small geographic distances.

Inbreeding

In populations where inbreeding is common, a heterozygote deficiency is observed compared to Hardy-Weinberg expected zygotic frequencies. All subpopulations in this study were found to be in Hardy-Weinberg equilibrium, thus indicating that inbreeding does not occur at any significant level in most populations of *Y.*

TABLE 3. PAIR-WISE COMPARISON OF HOMOGENEITY OF ALLELIC DISTRIBUTION BETWEEN SUBPOPULATIONS WITHIN EACH POPULATION. P-values that are bold indicate subpopulations which differ significantly in allele frequencies.

Pop.	Subpopulation		P-value
	1	2	
AZ	A	B	<0.01
	A	C	<0.01
	B	C	0.01
JT	A	B	0.03
	A	C	0.01
	B	C	<0.01
MP	A	B	0.46
	A	C	0.24
	B	C	0.55
UT	A	B	0.63
	A	C	0.43
	B	C	0.80
WM	A	B	0.12
	A	C	0.91
	B	C	0.37

brevifolia. It is still possible that self pollination events occur, but, perhaps the seeds are not viable as is the case in *Yucca filamentosa* (Marr et al. 2000).

Recruitment

A review of studies using Moran's I by Heywood (1991) indicated that selfing species show pronounced local genetic structure, while outcrossing species with random sexual reproduction and seed dispersal do not show any structured pattern. Vegetative reproduction would have an even stronger effect on population structure. Results of the spatial autocorrelation analysis indicate that genotypes in the 1-ha intensively sampled plot were randomly distributed, and thus that sexual reproduction through random mating, rather than vegetative reproduction or self-pollination, has been the predominant form of recruitment at this site.

Recruitment in *Yucca brevifolia* may be primarily episodic (Wallace and Romney 1972). A twenty-year study by Comanor and Clark (2000) of *Y. brevifolia* recorded no recruitment in their study areas. The combination of predominant outcrossing with long stretches of no measurable recruitment suggests that recruitment in *Y. brevifolia* is episodic, and likely reliant on rare environmental conditions. While this model of recruitment through outcrossing may be characteristic of this study plot, it may not be common for the species as a whole. Further, in this study an individual was defined as a stem or group of stems less than 1 m from one another. In many cases, the individuals sampled were composed of more than one stem. While vegetative reproduction appears to have a small role in recruitment of new individuals at this site, it may play a significant role in the longevity of individuals.

Further Research

This project has developed the first model of genetic structure found within populations of *Y. brevifolia* based on protein variation. Future studies investigating variation at the molecular level are needed to increase understanding of genetic variation both within and across populations. Through continued investigation into genetic structure of *Y. brevifolia*, biologists will develop a better understanding of the relationships of gene flow, genetic variation, and recruitment levels across the landscape of the Mojave Desert.

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